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## Obp4 and Act5C gene expression is related to Hygienic behavior in honeybee families

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## Abstract

Honeybees (*Apis mellifera*) evolved social immunity mechanisms based on coordinated behavioral mechanisms for reducing the spread of pathogens and parasites. In particular, Hygienic Behavior (HB) is an active participation of family defense by uncapping and removal of dead, diseased or parasitized brood (Cremer *et al.*, 2007). Currently, HB evaluation is performed only by in-field empirical assays, and suitable molecular markers that could allow the discrimination between families with High and Non-High HB score are not yet available. The research activity was characterized by a transversal approach: from in-field phenotypic characterization, to expression profiling of selected coding genes in honeybee brains. The expression analysis was performed on pools of 15 days-old honeybees collected in spring 2017 from 10 different colonies (5 with high HB and 5 with low HB scores). The brains were dissected and total RNA was purified. After quantitation and quality evaluation, the extracted RNAs were reverse transcribed to cDNAs and Real Time PCR was performed on different target genes (Act5C, Mblk-1, Obp3, Obp4, Obp16 and Obp18) previously showed by transcriptomic and proteomic studies to be involved in HB (Cristino *et al.*, 2014; Takeuchi *et al.*, 2001; Chandrasekaran *et al.*, 2011). The comparative analysis of RT-PCR threshold cycles (Ct) showed that Mblk-1 was the least expressed gene among the analyzed targets. Most importantly, statistical analysis of expression data validated ( $p < 0.05$ ) the delta-Ct between Obp4 and Act5C as a predictive index of high vs low HB score. These results confirmed the findings obtained through a preliminary experiment performed in spring 2016 (Dell'Orco *et al.*, 2016).

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